Review article

Ribozymes: an anti-viral agent

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Abstract

The discovery that RNA can act as an enzyme led Thomas Cech to win the Nobel Prize in Chemistry and led immediately to the next wave of attempts to find an effective RNA-based therapy. The tantalizing idea that RNA enzymes called trans-cleaving ribozymes enables them to act as potential antiviral and powerful tool for functional genomic studies. The efficacy of ribozyme function in a complex intracellular environment is dependent on the intracellular fate of the RNA that is being targeted. Recently, ribozymes have been used successfully to inhibit gene expression in a variety of biological systems in vitro and in vivo. Ribozyme has also been used successfully to combat many cases of viral infection, as clinical trial. Despite it needs to be investigated and explored as far as its structural and functional aspects are concern. In view of the significance of ribozyme in modern medicine, we reviewed the recent literature on general approach to control viral infection.

Keywords: ribozyme; molecular tool; gene therapy

INTRODUCTION

Ribozymes are best defined as RNA molecules with catalytic activity which may bind to and cleave RNA molecules in a sequence-specific manner. The development of trans-cleavage of ribozyme has resulted in an increasing number of reports using ribozymes for potentially therapeutic applications. Different types of catalytic RNAs exist viz hammerhead, hairpin, hepatitis delta virus (HDV) and Varkud Satellite (VS) RNA, group I and group II introns [1-3] and the RNA subunit of RNase P [4-6]. Moreover, ribosomal RNA is also considered a catalyst as revealed by structural and chemical analysis [7]. It was also found that RNA component of the spliceosome might also have enzymatic properties [8]. The discovery of ribozymes and fundamental studies on the mechanisms of self-splicing have revealed details on the catalytic core and the secondary and tertiary structure of RNA folding leading to ribozyme-mediated cleavage of RNA. Initially ribozymes were considered as metalloenzymes, requiring divalent metal ions for catalysis and that all ribozymes operate through a similar mechanism of action. However, recent efforts have been made to reveal the significant role of monovalent cations in RNA folding using Tetrahymena ribozyme as a model [9, 10]. Different types of ribozymes appear to exploit different cleavage mechanisms, which depend upon the structure of individual ribozymes.

Most recently ribozymes catalytic centers have been incorporated into antisense RNA and shown to specifically cleave a target RNA substrate. The enzymatic activity of the ribozyme catalytic center results in the cleavage and destruction of the target RNA. Pairing of ribozyme to the substrate needs to last long enough for the ribozyme to cleave the target RNA, causing functional inactivation of the target. Once the target is cleaved, the ribozyme can dissociate from the cleaved products and recycle itself through a repeat cycle of binding, cleavage and dissociation. The ability of ribozymes to cleave targets

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and then recycle themselves provides an advantage over standard antisense RNA which only inactivates the target RNA, without degrading it. This technology has started gaining ground in the applied bio-sciences and has a vast application in molecular medicine [11]

DESIGNING RIBOZYMES

Ribozymes are attractive potential therapeutic agents because of their specificity of binding and cleavage, potential for turnover and lack of immunogenicity. Here, we are summarizing the factors, which need to be considered in the designing of ribozymes, with special reference to anti-TET ribozymes.

Selection of target site is generally based on three criteria, biological significance of the target RNA, presence of an appropriate target, triplet sequence and accessibility of this sequence to ribozyme action [12, 13]. The biological significance of a target RNA must be assessed for each target gene. Several protocols have been developed to empirically determined, most appropriate target sites in RNA. These library selection technologies have shown clear utility and in vitro identification of sites has proven more effective for cellular application [12]. For hammerhead ribozyme cleavage, the target site is generally GUX, although in certain cases NUX may be used (where N represents any nucleotide and X represents A, C or U) [5]. Ribozyme recognition triplets may be identified within the target RNA, and accessibility assessed using RNA secondary structure analysis algorithms, or by empirical experiments such as chemical modification [14]. However, these methods provide only an approximation as to whether or not the target site is accessible since they do not take into account RNA tertiary structures or potential RNA-protein interactions, but they are still useful as a first step in the design of ribozymes (An anti-HIV ribozyme).

Another new system has been designed recently that allows the regulation of gene expression in response to an externally administered regulatory drug. This system is based on allosteric ribozymes, which can specifically cleave their own RNA in the absence of regulatory drug and can be inactivated in the presence of regulatory drug [12]. Thus, if such ribozymes were suitably inserted within mRNA transcripts, they would lead to its quick degradation, unless the regulatory drug is present. Such catalytic RNAs can be rationally designed using known ribozymes such as hammerhead ribozyme and regulatory sequences, called aptamers which are based on the riboswitch sensors. These sensors detect the co-factor of the aptazyme and suppress the gene expression with its anti-RBS sequence bound to RBS (ribosomal binding protein) of its own mRNA [15, 16]. Another molecule of cyclic nucleotide monophosphate was identified, which activates allosteric ribozymes at the concentration of 100 M. Upon activation the catalytic rates of these ribozymes were activated up to 5000-fold, and reached activities similar to the wild-type hammerhead ribozyme [17]. An in vitro selection scheme has also been applied to obtain allosteric ribozymes that respond to the antibiotic doxycyclin [18]. The level of inhibition was around 50-fold, while the response could be achieved at very low concentrations with inhibition constants as low as 20 nM.

RIBOZYME IN GENE THERAPY APPROACH AGAINST HIV

HIV infection is a relatively complicated series of events with pathologic events as well as drug-induced side effects. Several, gene-based approaches have been described for the inhibition of HIV-1 replication [19-27]. These include the expression of i) intracellular antibodies to viral proteins, [28, 29] antisense RNA, [21] RNA decoys such as RRE and TAR [30] to inhibit HIV-1 transcription and processing and v) ribozymes to catalytically cleave and thus inactivate HIV-1 RNA species [31-37].

Ribozymes act at several stages in the HIV infectious-cycle like the initial entry of genomic viral RNA into the target cell, transcription of genomic and subgenomic RNA molecules, prior to and during translation of mRNA to viral proteins, and prior to encapsidation of the genomic RNA. The cleavage of HIV RNA by ribozymes at any of these stages can lead to a decrease in intracellular viral replication (Figure 1)

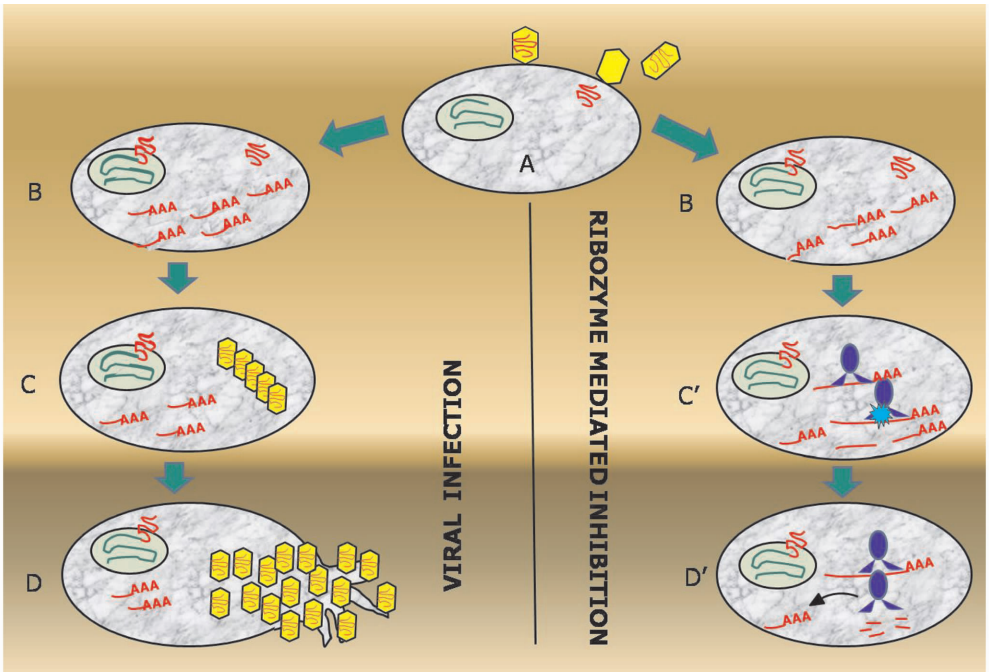

Figure. 1. Schematic representation of ribozyme inhibited and normal stages of viral infection. A: represents the attachment and infection of virus in native cell; B ,C & D depicts the ligation of virus genetic material to most genome, its transcription along with host genome, formation of active viral particle and its release from host cell on maturation, respectively. Panel C& D′represent the binding of ribozyme ( ) to mRNA and action of its catalytic domain leading to mRNA cleavage and also depicting the recycling of ribozyme due to its high turnover no.

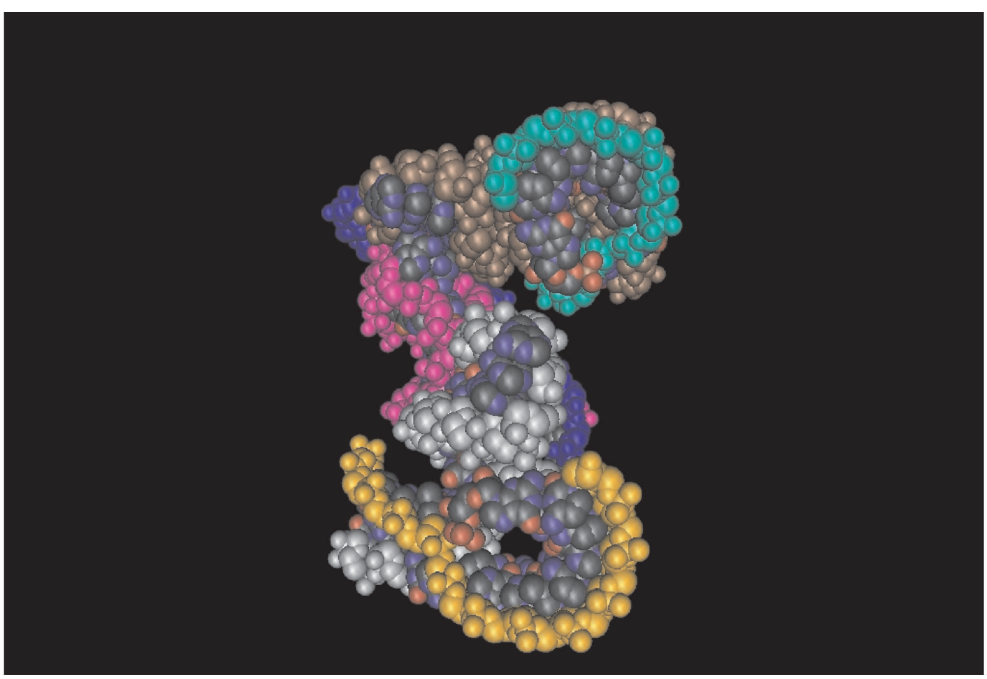

Figure. 2 Hammerhead ribozyme [52]. 3D structure of hammer head ribozyme prepared from 2.2Å structure of a full-length catalytically active hammerhead ribozyme [MMDB: 53274]. The figure is generated with the help of 4.1 version of Cn3D software. The meanings of these color ramps are explained as the follows: The highest lipophilic area of the molecule in brown, while it is the highest hydrophilic area in blue. (For interpretation of the references to color in this figure, the reader is referred to the web version of this software)

THE POTENTIAL APPLICATION OF RIBOZYMES TO THE TREATMENT OF AIDS

AIDS is a disease with a viral etiology and a marked immune component; the virus slowly destroys the cells of the immune system leading to immunodeficiency[39-45]. An effective treatment needs to address two facets of the disease; viral load and immune function. To date, therapies for the treatment of HIV-1 infection have focused on the reduction of viral load using drugs that interfere with replication of the virus with only a modest effect on the restoration of T-cell counts. Triple combination therapy (TCT), generally involving two reverse transcriptase inhibitors and one protease inhibitor, is the most recent and relatively successful development in the management of HIV infection [19-21]. Whilst TCT has shown great promise, often reducing to undetectable the level of HIV-1 in the blood, side effects are now being noticed including lipodystrophy, hyperlipidemia, and protease-related diabetes [22, 23]. In addition, strains of HIV are emerging which are resistant to both reverse transcriptase and protease inhibitors [23, 24]. New therapies which address the other aspect of HIV/AIDS disease management, namely immune restoration, need to be considered [25, 26].

Traditional anti-viral drugs for infectious disease are small molecules. However, recent advances in molecular biology have made gene-based therapies a possibility. As noted above, ribozymes are enzymatic molecules that can cut specific sequences in HIV and destroy the virus. Ribozymes cut HIV at several stages of its life cycle and are active against strains that are resistant to conventional anti-viral therapy. Ribozyme gene therapy could be used as an adjunctive or stand-alone therapy, and is potentially cost-competitive with other anti-viral therapies.

ANTI-HIV RIBOZYME UNDER PHASE I CLINICAL TRIAL

Clinical trials are currently being conducted by several groups to address questions, whether ribozymes can impact on AIDS disease course, and the two surrogate markers of advancing disease, viral load and CD4 + T-cell counts, is about to be assessed. Two independent Phase I clinical trials were initiated to test the safety and feasibility of an anti-HIV-1 ribozyme gene therapeutic approach. The secondary aims of the studies are to assess the ability to detect ribozyme-containing cells in the bloodstream. Both clinical trials utilize the LNL6 vector and the recombinant RRz2. The two trials used different target cell populations: CD4 + PBL or CD34 + stem cells. The first Phase I clinical trial involved identical twins, discordant for infection with HIV. Healthy CD4 + PBL from the uninfected twin were transduced with either LNL6 or RRz2 and both cell populations cultured and expanded ex vivo [45] before infusion into the bloodstream of the corresponding HIV-positive twin. The patients were then monitored for signs of any adverse events, and assessed for CD4 + lymphocyte counts, HIV-1 viral load, and presence of RRz2 and LNL6 vector in peripheral blood lymphocytes. The second Phase I clinical trial involved the removal, transduction and infusion of CD34 + blood stem cells within HIV-positive individuals. As in the CD4 + -based trial, two populations of marked cells were imparted (LNL6 and RRz2-transduced cells). The rationale for this trial is that these transduced CD34 + stem cells will home and reconstitute in the bone marrow compartment, with subsequent proliferation and differentiation to give rise to a variety of hematopoietic lineages including CD4 + T-lymphocytes and macrophages. The patients were monitored for signs of any adverse events, and assessed for CD4 + lymphocyte counts, HIV-1 viral load, and presence of RRz2 and LNL6 vector in bone marrow, and purified peripheral blood lymphocytes, monocytes and granulocytes. In each of the trials, separate populations of cells were transduced with either retroviral vector containing the ribozyme (RRz2) or the vector alone (LNL6), the latter as an internal control. Approximately equal numbers of the two transduced cell types were then introduced into the recipient patients. This allowed monitoring of the survival of anti-HIV-1 ribozyme-expressing cells. Cell survival is monitored by simultaneously detecting ribozyme and control vector DNA sequences in peripheral blood using a semi-quantitative PCR procedure [45]. In order to examine the ability of the CD34 + stem cells to repopulate multiple cell lineages, PCR is performed on bone marrow, and purified cell populations.

RIBOZYME AGAINST HEPATITIS INFECTION

Hepatitis C virus (HCV), an RNA virus is the major infectious agent responsible for chronic hepatitis. No vaccine has yet been developed to protect against the disease caused by HCV. To investigate new genetic approaches in order to control this infection, six hammerhead ribozymes have been designed which can bind on the conserved region of the plus strand and minus strand of the HCV genome. Moreover, these potential ribozyme molecules were characterized and expressed using recombinant adenovirus vectors. The expressed ribozyme plus or minus strand HCV RNA were expressed in cell culture and primary human hepatocytes obtained from chronic HCV-infected patients. To investigate the potential use of synthetic stabilized ribozymes for the treatment of chronic hepatitis C virus (HCV) infection, Wang et al [14] determine the advantage of U1 small nuclear RNA as ribozyme vector (U1-R2) to inhibit HCV replication in vivo. Third stem loop was replaced by HCV core specific ribozyme to construct as U1-R2 plasmid which can express in eukaryotic system. These plasmids were co-transferred with pCMV/T7-NCRC Delta-luc into Huh7 cell lines using lipofectin. A U1 SnRNA chimeric ribozyme was constructed successfully. Luciferase expression in Huh7 was found to be suppressed by R2 and U1-R2 by 48.64% and 87.46% respectively [14]. The notion of using ribozymes as therapeutic agents is receiving increasing attention by pharmaceutical research. Over the past several years, a good number of clinical trials have been initiated to evaluate the potency of ribozymes for treating a wide range of infection cases, including HCV [46]. Hepatitis B virus (HBV) infection is major public health problem, associated with cirrhosis and hepatocellular carcinoma, and has thus become a major problem. It was estimated that, approximately 2 billion people are infected with HBV worldwide and about 400 million are HBV chronic carriers [47]. Hammerhead ribozymes (Fig 2) have recently gained some attention as potential tools to inhibit viral infection. As opposed to an ex vivo ribozyme gene transfer strategy in AIDS patients, hepatitis B virus may be susceptible to the injection of synthetic ribozymes or to in vivo transfer of ribozyme expression vectors. Hepatitis delta virus (HDV) can be used as a vector to deliver biologically active RNA into hepatocytes. It delivers hammerhead ribozyme into hepatocytes [48]. The system provides a new approach for the study of mechanisms of HBV replication as well as potential treatment of HBV infection [49, 50].

CONCLUSION

Despite of significant progress over the decades, treatment of viral infection still remains as a big challenge. Ribozyme could be selected as a tool to contend with viral diseases, which seems quite possible with the advent of modern technologies. The major problem which comes across is improper delivery system for ribozyme due to its large molecular size. An inclination toward RNAi; as a strong tool against viral infection has became a best substitute for ribozyme. Inspite of all ribozyme has its own merits in the modern medicine. The Fundamental researches on structural and functional relationship of ribozyme at academic as well as industrial level are in progress. This has become an important area to understand the insight of the cellular activities of ribozyme inside the cell. This will definitely put a big impact on both diagnostic and therapeutic methodology among the human and veterinary medicine in the near future.

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